

BEST AVAILABLE COPY

D4



(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

SCIENCE REFERENCE LIBRARY

(11) Publication number

D10
0130523
A2

(12)

EUROPEAN PATENT APPLICATION

(21) Application number: 84107266.3

(51) Int. Cl.: C 12 Q 1/68

(22) Date of filing: 25.06.84

// G01N33/548, G01N33/547

(30) Priority: 05.07.83 US 511064
18.05.84 US 611667

(71) Applicant: Molecular Diagnostics, Inc., 400 Morgan Lane, West Haven, CT.06516 (US)

(43) Date of publication of application: 09.01.85
Bulletin 85/2

(72) Inventor: Dattagupta, Nanibhushan, 470 Prospect Street, New haven, Ct 06511 (US)
Inventor: Crothers, Donald M., Surrey Drive, Northford, Ct 06472 (US)

(84) Designated Contracting States: CH DE FR GB IT LI NL SE

(74) Representative: Adrian, Albert, Dr. et al, c/o BAYER AG Konzernverwaltung RP Patentabteilung, D-5090 Leverkusen 1, Bayerwerk (DE)

(54) Immobilized nucleic acid probe and solid support for nucleic acids.

(57) A solid support capable of binding a nucleic acid thereto upon suitable irradiation, comprising (a) a solid substrate, (b) a photochemically reactive intercalator compound or other nucleic acid-binding ligands, and (c) divalent radical chemically linking the substrate and the ligand (b). Specifically, a hydroxy group-containing solid substrate such as nitrocellulose paper is linked via a bifunctional reagent such as cyanogen bromide or 1,4-butanediol-diglycidyl ether to an amino-substituted angelicin or psoralen or ethidium bromide which in turn is photochemically linked to a nucleic acid. The resulting immobilized nucleic acid probe is capable of hybridizing with complementary nucleic acid fragments and is thereby useful in diagnostic assays.

EP 0130523 A2

0130523

IMMOBILIZED NUCLEIC ACID PROBE AND SOLID SUPPORT FOR
NUCLEIC ACIDS

The present invention relates to a novel way of joining a nucleic acid to a solid substrate so as to provide a solid probe suitable for use in various tests, particularly hybridization assays for the determination of specific polynucleotide sequences.

In DNA-DNA hybridization and DNA-RNA hybridization assays, one of the complementary nucleic acid chains is commonly coupled to a solid support. This helps to reduce the background and can be used to separate or isolate the corresponding nucleic acid. The methods of attachment of DNA to a solid support have involved (1) non-specific physical adsorption of a single-stranded DNA to nitrocellulose papers, and (2) covalent attachment via diazo coupling. Both methods are specific for single-stranded DNA. These covalent reactions are non-specific and several sites are coupled. These cause ineffective hybridization and loss of perfect fidelity. Several points of attachment per chain reduces the flexibility of the DNA and reduces the rate of hybridization. Moreover, the lifetime of such an adduct is not very long. The DNA comes off easily and it is difficult to quantify the amount on the solid support, without the use of radioactivity. The use of DNA probes for diagnostic purposes demands an effective method of tagging the DNA to a phase which can be separated easily from the rest of the nucleic acids.

U.S. Application Serial No. 511,063, filed on July 5, 1983, now pending in the names of Dattagupta, Rae and Crothers discloses various tests for nucleic acids, e.g., DNA of individuals being tested for sickle cell anemia. The test involves a soluble labelled probe and a probe fixed to a solid support. The probe can be fixed to the support chemically as by a bifunctional reagent which at one end reacts with the support, e.g., a hydroxyl group of a cellulose molecule, and at the

0130523

other end reacts with the DNA. This is quite satisfactory for many purposes but in some instances there may be too much bonding between the substrate and DNA, impairing the sensitivity of the DNA in the test.

5 It is accordingly an object of the present invention to provide a way of binding a nucleic acid to a solid substrate easily and without impairing the sensitivity of the DNA in the test.

10 These and other objects and advantages are realized in accordance with the present invention wherein there is provided a solid support capable of binding a nucleic acid thereto upon suitable irradiation, comprising (a) a solid substrate, (b) a photochemically reactive nucleic acid-binding ligand, and (c) a divalent radical 15 chemically linking the substrate and the nucleic acid-binding ligand.

20 The specific coupling reagents employed are functionalized, photochemically reactive nucleic acid-binding ligands, e.g., intercalator compounds such as amino-substituted furocoumarins, e.g., amino-methyl-dimethyl-angeticin and amino-methyl-trimethyl-psoralen, and aminophenanthridium halides as well as closely related chemical derivatives thereof, and non-intercalator compounds such as netropsin, distamycin, Hoechst 25 33258 and bis-benzimidazole. Upon photoactivation these reagents will chemically link with nucleic acids. These reagents have a functionalized site other than the nucleic acid-reactive site and, by such other site, they are joined to a solid substrate, thereby in turn joining 30 the nucleic acid to such substrate with a minimum impairment of the nucleic acid function.

35 Apparently functionalized and photochemically reactive forms of a wide variety of intercalating agents can be used as the coupling reagent as exemplified in the following table:

0130523

Intercalator Classes and Representative Compounds

Literature References

- | | | |
|----|--|--|
| 5 | A. Acridine dyes | Lerman, J. Mol. Biol. 3:18(1961); Bloomfield et al, "Physical Chemistry of Nucleic Acids", Chapter 7, pp. 429-476, Harper and Rowe, NY(1974)
Miller et al, Bio-polymers 19:2091(1980) |
| 10 | B. Phenanthridines | Bloomfield et al, supra;
Miller et al, supra |
| 15 | ethidium
coralyne | Wilson et al, J. Med. Chem. 19:1261(1976) |
| 20 | ellipticine, ellipticine cation and derivatives | Festy et al, FEBS Letters 17:321(1971);
Kohn et al, Cancer Res. 35:71(1976); LePecq et al, PNAS (USA)71: 5078(1974); Pelaprat et al, J. Med. Chem. 23:1330(1980) |
| 25 | C. Phenazines
5-methylphenazine cation | Bloomfield et al, supra |
| | D. Phenothiazines
chloropramazine | ibid |
| | E. Quinolines
chloroquine
quinine | ibid |
| | F. Aflatoxin | ibid |
| 30 | G. Polycyclic hydrocarbons and their oxirane derivatives | ibid |
| | 3,4-benzpyrene,
benzpyrene diol
epoxide, 1-pyrenyl-oxirane | Yang et al, Biochem. Biophys. Res. Comm. 82:929(1978) |
| 35 | benzanthracene-5,6-oxide | Amea et al, Science 176:47(1972) |

0130523

Bloomfield et al, supra

- H. Actinomycins
actinomycin D
- I. Anthracyclinones
 β -rhodomycin A
daunamycin
- 5 J. Thiaxanthenones
miracil D
- K. Anthramycin
- L. Mitomycin
- 10 M. Platinum Complexes
- 15 N. Polyintercalators
echinomycin
- 20 quinomycin
triostin
BBM928A
tandem
- 25 diacridines
- 30
- 35 ethidium dimer
- ibid
- ibid
- ibid
- Ogawa et al, Nucl. Acids Res., Spec. Publ. 3:79(1977); Akhtar et al, Can. J. Chem. 53:2891(1975)
- Lippard, Accts. Chem. Res. 11:211(1978)
- Waring et al, Nature 252:653(1974); Wakelin, Biochem. J. 157:721(1976)
- Lee et al, Biochem. J. 173:115(1978); Huang et al, Biochem. 19: 5537(1980); Viswamitra et al, Nature 289: 817(1981)
- LePecq et al, PNAS (USA) 72:2915(1975); Carrellakis et al, Biochim. Biophys. Acta 418:277(1976); Wakelin et al, Biochem 17:5057(1978); Wakelin et al, FEBS Lett. 104:261(1979); Capelle et al, Biochem. 18:3354(1979); Wright et al, Biochem. 19:5825(1980); Bernier et al, Biochem. J. 199:479 (1981); King et al, Biochem. 21:4982(1982)
- Gaugain et al, Biochem. 17:5078(1978); Kuhlman et al, Nucl. Acids Res.

5:2629(1978); Maricovits et al, Anal. Biochem. 94:259(1979); Dervan et al, JACS 100:1968(1978); ibid 101:3664(1979).

5	ellipticene dimers and analogs	Debarre et al, Compt. Rend. Ser. D. 284: 81(1977); Pelaprat et al, J. Med. Chem. 23:1336(1980)
10	heterodimers	Cain et al, J. Med. Chem. 21:658(1978); Gaugain et al, Biochem. 17:5078(1978)
15	trimers	Hansen et al, JCS Chem. Comm. 162(1983); Atnell et al, JACS 105:2913(1983)
O.	Norphillin A	Loun et al, JACS 104: 3213(1982)
P.	Fluorenes and fluorenones fluorenodiamines	Bloomfield et al, supra Witkowski et al, Wiss. Beitr.-Martin- Luther-Univ. Halle Wittenberg, 11(1981)
Q.	Furocoumarins angelicin	Venema et al, MGG, Mol. Gen. Genet. 179;1 (980)
25	4,5'-dimethyl angelicin	Vedaldi et al, Chem.- Biol. Interact. 36: 275(1981)
psoralen	Marciani et al, Z. Naturforsch B 27(2): 196(1972)	
30	8-methoxysoralen	Belognzov et al, Mutat. Res. 84:11(1981); Scott et al, Photochem. Photobiol. 34:63(1981)
35	5-aminomethyl-8- methoxysoralen	Hansen et al, Tet. Lett. 22:1847(1981)
	4,5,8-trimethylpsoralen	Ben-Hur et al,

0130523
Biochim. Biophys.
Acta 331:181(1973)

4'-aminomethyl-4,5,8-trimethylpsoralen

Issacs et al, Biochem.
16:1058(1977)

5 xanthotoxin

Beaumont et al,
Biochim. Biophys.
Acta 608:1829(1980)

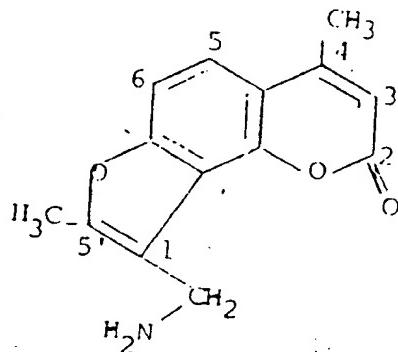
R. Benzodipyrone

Murx et al, J. Het.
Chem. 12:417(1975);
Horter et al, Photo-
chem. Photobiol. 20:
407(1974)

S. Monostral Fast Blue

Juarranz et al, Acta
Histochem. 70:130 (1982)

15 Angelicin, more accurately 4'-aminomethyl-4,5'-dimethylangelicin, has the structural formula

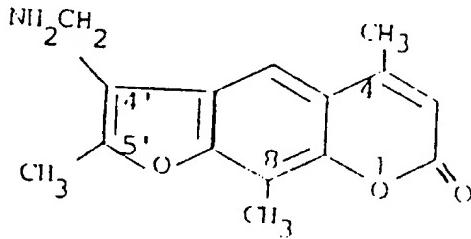


20

(see Dall'Acquz et al, Photochemistry and Photobiology, Vol. 37, No. 4, pp. 373-379, 1983.)

25 Psoralen, more accurately 4'-aminomethyl-4,5',8-tri-methyl-psoralen (AMT), has the structural formula

30



35

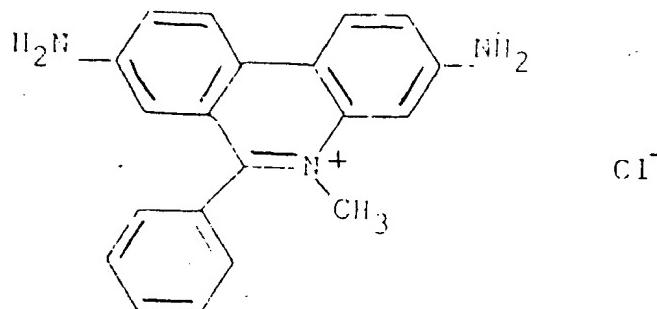
10130523

(Cadet et al, Photochemistry and Photobiology, Vol. 37,
No. 4, pp. 363-371, 1983.)

Methidium chloride, for example, has the formula

5

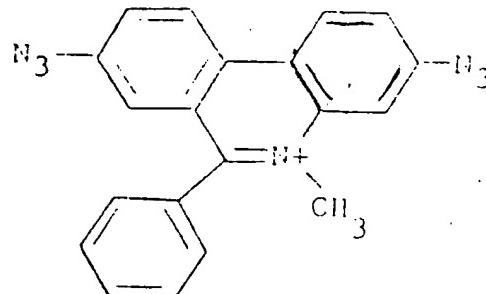
10



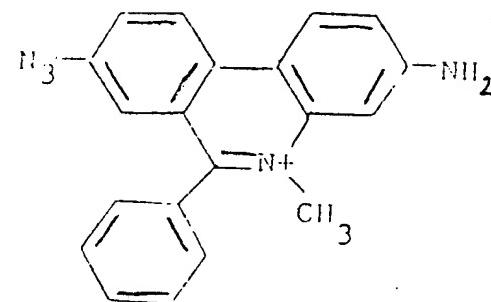
(see Graves et al, Biochemistry, 1981, Vol. 20 pp.
15 1887-1892.) Its mono- and di-azide analogues, shown
below, are comparably reactive:

20

25



methidium monoazide



methidium diazide

30

as are the ethyl counterparts and the 4-(3-aminopropyl-N-carbamoyl) derivative of the phenyl side chain
(methidium propylamine).

35

The solid substrate can be any solid which has reactive groups which could be carboxyl, amino or the like, but the preferred reactive groups are hydroxyl such as are found on cellulose. The cellulose may be unmodified as in cotton or paper or regenerated as in rayon or partially esterified as in cellulose acetate, cellulose propionate and especially cellulose nitrate,

0130523

or partially etherified as in methylcellulose and carboxymethylcellulose.

While the photochemically active intercalator reagent could be directly combined with the solid substrate, advantageously there is a mutual coupler which makes the connection. Suitable reagents include bifunctional compounds such as cyanogen bromide (CNBr), 1,4-butanediol diglycidyl ether, and the like. These are reacted with both the solid substrate and the photochemical reagent simultaneously or first with one and then with the other.

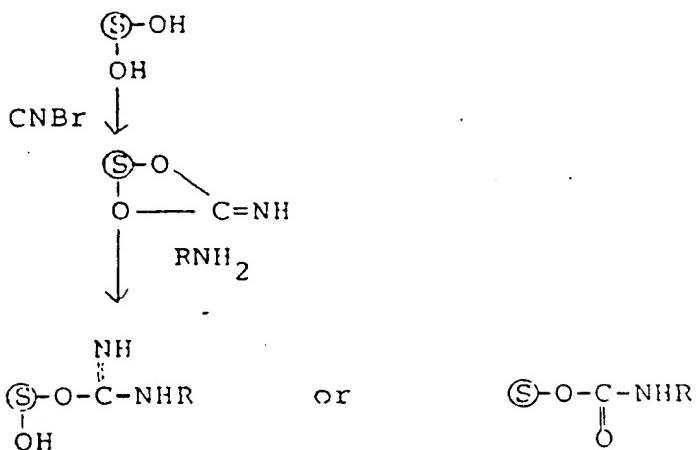
Thereafter, the product is further reacted with the nucleic acid photochemically. The reactions with the coupler and nucleic acid are substantially quantitative so the quantities of the reagents employed depend upon the desired ratio of nucleic acid to solid support. For most purposes, about 0.1 to 1000 mg and preferably about 1 to 100 mg of nucleic acid per gram of solid support will be suitable, although it may be higher or lower, depending upon the molecular weight of the nucleic acid, its sensitivity and the particular test in which it is to be used.

The reaction conditions in each step are generally known per se and any solvents and temperatures can be employed which permit the reactions to proceed without interference, e.g., from about -10 to 100°C., preferably about 10 to 50°C., and most preferably room temperature, employing inert organic solvents such as ether, carbon tetrachloride, THF, and the like.

The photochemically active reagents herein employed preferably react through amino groups. Identifying it as RNH_2 and the substrate with pendent OH groups as S, the stepwise reactions are as follows:

10 0130523

A. a) with CNBr



b) S-OH

$$\begin{array}{c} \text{CH}_2-\underset{\text{O}}{\text{CH}}-\text{CH}_2-\text{O}-\text{(CH}_2\text{)}_4-\text{O-CH}_2-\underset{\text{O}}{\text{CH}}-\text{CH}_2 \\ | \qquad \qquad \qquad | \\ \text{S-O-CH}_2-\underset{\text{OH}}{\text{CH}}-\text{O}-\text{(CH}_2\text{)}_4-\text{O-CH}_2-\underset{\text{O}}{\text{CH}}-\text{CH}_2 \\ | \\ \text{RNH}_2 \\ \downarrow \\ \text{S-O-CH}_2-\underset{\text{OH}}{\text{CH}}-\text{O}-\text{(CH}_2\text{)}_4-\text{O-CH}_2-\underset{\text{OH}}{\text{CH}}-\text{CH}_2-\text{NHR} \end{array}$$

B. (S)  NIR

25 nucleic acid ↓ irradiation

 ⑤ Nucleic acid
 Amino-derivatives of angelicin and psoralen react correspondingly, if not identically.

30 The particular wavelength of radiation selected
will depend upon the particular photoreagent and
whether it is desired to bind to a single strand of
nucleic acid or to a double strand. If to both strands
it can be in a manner and to a degree such that the
35 nucleic acid is no longer denaturable.

01301523

The nucleic acid can be RNA or DNA or short (oligonucleotide) or long chain length, as desired, doubly or singly stranded.

5 Formation of monoadducts is desirable for hybridization experiments. In crosslinks, both DNA strands are covalently linked to psoralen chromophore and hence strand separation prior to hybridization is difficult. If the probe to be hybridized is linked to another non-specific piece of DNA, the non-specific part can be linked either via crosslink or monoadduct formation. In this case, irradiation can be done at any wavelengths between 300-390 nm. Irradiation at 390 nm produces monoadduct; irradiation at 360-300nm produces both monoadduct and crosslinks.

10 15 If angelicin compounds are used, the product will predominantly be monoadduct irrespective of the wavelength of irradiation.

20 The invention will now be further described with reference to the accompanying examples wherein all parts are by weight unless otherwise expressed.

Example:

1. Activation of the solid support and coupling of AMT.

25 The procedure described below has been followed for Sephadex G25 and cellulose, but any hydroxy-containing solid support can be activated by an identical procedure.

30 a) Activation with 1,4-butane-diol-diglycidyl ether.

35 0.5-1 gm solid powder is swollen with water and washed, then 5-10 ml sodium hydroxide solution (0.5 M) is added. To this thick suspension, 1 ml 1,4-butanediol-diglycidyl ether is added. The suspension is shaken overnight on a mechanical shaker and then washed with sodium hydroxide (0.5 M) solution and 1.0 ml 4'-aminomethyl-4,5',8-trimethyl-psoralen (2 mg/ml) in water is added, followed by enough 1 M sodium

013-0523

hydroxide to have a thick suspension. The suspension is then stirred gently for 24 hours at room temperature and excess unreacted residues are quenched with lysine.

5 The solid is then washed with water followed by the desired aqueous buffer solution for DNA coupling.

10 b) For epoxidation of paper the identical procedure is followed with Whatman filter papers type 540, 1 and 541. The filter papers are taken on a watch glass or beaker cover (glass) and turned occasionally by hand. The rest of the procedure is the same as above.

15 c) Activation by cyanogen bromide and coupling of AMT. Typical example with cellulose:

20 0.5 gm cellulose is swollen in 5.0 ml distilled water for one hour. The swollen gel is washed thoroughly with distilled water. Then it is taken in an erlenmeyer flask, ice-cooled distilled water is added to the swollen cellulose and the pH is adjusted between 10.5 - 11 with 5M sodium hydroxide solution.

25 The flask with its contents is cooled in ice to avoid temperature rise above 15°C. 1 gm of solid cyanogen bromide is added to the cellulose and the solution is stirred for 30 minutes and pH maintained between 10.5 - 11 by NaOH. The suspension is washed with ice cold distilled water, water is removed by centrifugation and 20 ml ice cold potassium phosphate buffer (10 mM; pH 8) is added. The activated cellulose is kept in brown bottles (in small aliquots) at - 20°C.

30 2 - 3 ml of swollen, activated gel is taken in a brown bottle and 0.7 ml AMT (2mg/ml) is added and the mixture is shaken gently in the cold room. Excess activated residues are quenched with lysine. The solid is washed with aqueous buffer for DNA binding.

35 d) For papers, similar procedures have been followed with Whatman cellulose filter papers type 540, 1 and 541 quantitative papers. Care should be taken to avoid tearing of the papers.

U130523

e) Parallel experiments with ^3H labelled aminomethylpsoralen or angelicin are used to estimate labelling efficiency.

2. Coupling of phenanthridium compounds to a solid support and azide formation for photochemical coupling of DNA:

Activation of the solid supports is done by the method described above. As an example, methidium propylamine (R.P. Hertzberg and P.B. Dervan, JACS, 104, 10 313 (1982)) is coupled to the solid support, using identical buffer conditions as in 1. The isolated methidium containing solid support is then diazotized and azide derivative is made as follows. 1 gm cellulose or ($2 \times 5 \text{ cm}^2$) of a sheet of activated paper 15 containing methidium chloride is taken in 20 ml water, cooled in ice, 0.2 ml ice cold HCl is added; sodium azide (20 mg solid; 2x) is added. The vessel is cooled in ice and sodium nitrate solid (100 mg) is added. The reaction is allowed to proceed for 30 minutes, solid 20 support is washed with the desired buffer. Coupling of DNA and hybridization are carried out the same way as described for aminomethyl-psoralen. Aminomethyl-dimethyl-angelicin can be similarly treated.

3. Photochemical coupling of DNA:

25 0.5 ml (0.2 - 0.3 gm gel + buffer) activated solid powder or $0.8 \times 1 \text{ cm}^2$ activated paper is taken in a 1 cm path length spectrophotometer cuvette. Adenovirus DNA (partially labelled with ^3H) (concentration 25 $\mu\text{g/ml}$) in tris EDTA buffer (10 mM tris, 1mM EDTA, pH 7.5) is added to the cuvette and irradiation is done at a desired wavelength for 30 minutes to two hours depending on the future needs. For AMT, irradiation at 390 nm produces monoadduct whereas at 360 - 300 nm both monoadduct and crosslinks are formed. By altering the 30 concentration and DNA sequence, crosslink to monoadduct formation can be modulated. After photoirradiation, the solid is washed and the radioactivity of the

washings and the solid support is counted in a Beckman 7800 scintillation counter.

Typical Results

		DNA	
	<u>Solid support</u>	<u>% Coupling</u>	<u>Coupled µg</u>
5	0.5ml or 0.8 x 1 control paper (No DNA)	-	-
10	BDGE treated paper	80	20
15	Cellulose cellex CNBr activated	91.5	22.5
20	Cellulose cellex BDGE activated	93.4	22.5
	Sephadex G25 CNBr activated	69.5	18.0

4. Assay for DNA-DNA hybridization of DNA photochemically coupled to the solid support:
 Andenovirus DNA is covalently coupled to the solid support as above and hybridization with ^3H labelled adenovirus DNA is done following the procedure of Noyes and Stark, Cell, 5, 301-310 (1975).
5. Use of photochemically coupled DNA for sickle cell diagnosis: AMT coupled DNA can be recovered as free DNA by irradiation at 260 nm. The separation probe (Application Serial No. 511,063, supra) is coupled to the solid support by the method described above. Then the support with the coupled DNA is mixed with the unknown and the detection probe under hybridization condition - as in 4. The solid support is then tested for the presence of label. If a radactively labelled detection probe is used, radioactivity is counted.

5a. The product of 4 is irradiated at 260 nm in otherwise the same manner as in 3, whereupon the DNA uncouples from the solid support, entering the solvent medium, viz. aqueous buffer. Then the liquid is assayed for ^{3}H .

It will be understood that the specification and examples are illustrative but not limitative of the present invention and that other embodiments within the spirit and scope of the invention will suggest themselves to those skilled in the art.

15

20

25

30

35

CLAIMS:

1. A solid support capable of binding a nucleic acid thereto upon irradiation, comprising (a) a solid substrate, (b) a photochemically reactive nucleic acid-binding ligand, and (c) a divalent radical chemically linking the substrate and the nucleic acid-binding ligand.
- 5 2. A support according to claim 1, characterized in that the nucleic acid-binding ligand is an intercalator compound selected from acridine dyes, phenanthridines, phenazines, furocoumarins, phenothiazines, and quinolines.
- 10 3. A support according to claim 1 or 2, characterized in that the substrate (a) in free state has free OH groups through which it is linked by the divalent radical (c).
- 15 4. A support according to any of claims 1 to 3, characterized in that the substrate (a) is cellulose or a cellulose ester.
- 20 5. A support according to any of claims 1 to 4, characterized in that the divalent radical (c) is derived from cyanogen bromide or from 1,4-butanediol-diglycidyl ether.
- 25 6. A support according to any of claims 1 to 5, characterized in that (b) is psoralen, angelicin, ethidium or derivatives thereof.
7. An immobilized nucleic acid probe comprising (a) a nucleic acid, (b) a nucleic acid-binding ligand photochemically linked to the nucleic acid, and (c) a

0130523

solid substrate chemically linked through a divalent radical to the nucleic acid-binding ligand (b).

8. An immobilized probe according to claim 7,
characterized in that the nucleic acid-binding ligand
5 is an intercalator compound selected from acridine dyes,
phenanthridines, phenazines, furocoumarins, phenothiazines,
and quinolines.

9. An immobilized probe according to claim 7 or
10 8, characterized in that the substrate (c) in free state
has free OH groups through which it is linked by the
divalent radical.

10. An immobilized probe according to any of
claims 7 to 9, characterized in that (b) is psoralen,
angelicin, ethidium or derivatives thereof.



Europäisches Patentamt

European Patent Office

Office européen des brevets

SCIENCE REFERENCE LIBRARY

(11) Publication number:

0 130 523

A3

(12)

EUROPEAN PATENT APPLICATION

(21) Application number: 84107266.3

(51) Int. Cl.: C 12 Q 1/68

//G01N33/548, G01N33/547

(22) Date of filing: 25.06.84

(30) Priority: 05.07.83 US 511064
18.05.84 US 611667

(71) Applicant: Molecular Diagnostics, Inc.
400 Morgan Lane
West Haven, CT 06516(US)

(43) Date of publication of application:
09.01.85 Bulletin 85/2

(72) Inventor: Dattagupta, Nanibhushan
470 Prospect Street
New Haven, Ct 06511(US)

(88) Date of deferred publication of search report: 23.07.86

(72) Inventor: Crothers, Donald M.
Surrey Drive
Northford, Ct 06472(US)

(84) Designated Contracting States:
CH DE FR GB IT LI NL SE

(74) Representative: Adrian, Albert, Dr. et al,
c/o BAYER AG Konzernverwaltung RP Patentabteilung
D-5090 Leverkusen 1, Bayerwerk(DE)

(54) Immobilized nucleic acid probe and solid support for nucleic acids.

(57) A solid support capable of binding a nucleic acid thereto upon suitable irradiation, comprising (a) a solid substrate, (b) a photochemically reactive intercalator compound or other nucleic acid-binding ligands, and (c) divalent radical chemically linking the substrate and the ligand (b). Specifically, a hydroxy group-containing solid substrate such as nitrocellulose paper is linked via a bifunctional reagent such as cyanogen bromide or 1,4-butanedioldiglycidyl ether to an amino-substituted angelicin or psoralen or ethidium bromide which in turn is photochemically linked to a nucleic acid. The resulting immobilized nucleic acid probe is capable of hybridizing with complementary nucleic acid fragments and is thereby useful in diagnostic assays.

A3

EP 0 130 523



**European Patent
Office**

EUROPEAN SEARCH REPORT

0130523
Application number

EP 84 10 7266

DOCUMENTS CONSIDERED TO BE RELEVANT

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl 4)
A	CHEMICAL ABSTRACTS, vol. 88, no. 11, 13th March 1978, page 99, no. 69689r, Columbus, Ohio, US; L. KITTLER et al.: "Photochemically induced binding of furocoumarins with lambda phage DNA in situ", & STUD. BIOPHYS. 1977, 66(3), 237-41 * Abstract *	1, 2	C 12 Q 1/68 G 01 N 33/548 G 01 N 33/547
A	---		
A	US-A-4 169 204 (J. HEARST et al.) * Abstract; column 1 *	1, 2	
A	---		
A	CHEMICAL ABSTRACTS, vol. 86, no. 19, 9th May 1977, page 118, no. 134373h, Columbus, Ohio, US; P. CHANDRA: "Nucleic acid modification by furocoumarins and light: some biomedical implications", & PHOTOCHEMOTHERAPIE, VERHANDLUNGSBER. DTSCH.-SCHWED. SYMP. PHOTOMEDIZIN 1975 (Pub. 1976, 25-32 * Abstract *	1, 2	TECHNICAL FIELDS SEARCHED (Int. Cl 4)
	---	-/-	C 12 Q G 01 N

The present search report has been drawn up for all claims

Place of search THE HAGUE	Date of completion of the search 23-04-1986	Examiner OSBORNE H.H.
-------------------------------------	---	---------------------------------

CATEGORY OF CITED DOCUMENTS

- X : particularly relevant if taken alone
 Y : particularly relevant if combined with another document of the same category
 A : technological background
 O : non-written disclosure
 P : intermediate document

- T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons
& : member of the same patent family, corresponding document



European Patent
Office

EUROPEAN SEARCH REPORT

0T30523

Application number

EP 84 10 7266

DOCUMENTS CONSIDERED TO BE RELEVANT

Page 2

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
A	CHEMICAL ABSTRACTS, vol. 98, no. 9, 28th February 1983, page 315, no. 68377g, Columbus, Ohio, US; H. BUENEMANN: "Immobilization of denatured DNA to macroporous supports : II. Steric and kinetic parameters of heterogeneous hybridization reactions", & NUCLEIC ACIDS RES. 1982, 10(22), 7181-96 * Abstract *	1,2,5	
A	---	1,2	
A	CHEMICAL ABSTRACTS, vol. 96, no. 7, 15th February 1982, page 207, no. 47770y, Columbus, Ohio, US; R.P. HERTZBERG: "Cleavage of double helical DNA by methidium-propyl-EDTA-iron(II), & J. AM. CHEM. SOC. 1982, 104(1), 313-15 * Abstract *		TECHNICAL FIELDS SEARCHED (Int. Cl. 4)
A	---	1,2	
	---	-/-	
The present search report has been drawn up for all claims			
Place of search	Date of completion of the search	Examiner	
THE HÄGUE	23-04-1986	OSBORNE H.H.	

CATEGORY OF CITED DOCUMENTS

- X particularly relevant if taken alone
- Y particularly relevant if combined with another document of the same category
- A technological background
- O non-written disclosure
- P intermediate document

- T theory or principle underlying the invention
- E earlier patent document, but published on, or after the filing date
- D document cited in the application
- L document cited for other reasons
- & member of the same patent family, corresponding document



European Patent
Office

EUROPEAN SEARCH REPORT

0130523
Application number

EP 84 10 7266

Page 3

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.)
A	CHEMICAL ABSTRACTS, vol. 96, no. 5, 1st February 1982, page 310, no. 31231n, Columbus, Ohio, US; & DD - A - 148 955 (AKADEMIE DER WISSENSCHAFTEN DER DDR, ZENTRALINSTITUT FUER MOLEKULARBIOLOGIE) 17-06-1981 * Abstract *	1-4	
A	EP-A-0 070 687 (STANDARD OIL CO.) * Page 4, paragraph 2; page 6, line 20 - page 7, line 14 *	1-4	
-----			TECHNICAL FIELDS SEARCHED (Int. Cl.)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	23-04-1986	OSBORNE H.H.
CATEGORY OF CITED DOCUMENTS		
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		
T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document		

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- BLACK BORDERS**
- IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- FADED TEXT OR DRAWING**
- BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- SKEWED/SLANTED IMAGES**
- COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- GRAY SCALE DOCUMENTS**
- LINES OR MARKS ON ORIGINAL DOCUMENT**
- REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.